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Hormone Discharges from a Midwest Tile-Drained Agroecosystem Receiving Animal Wastes

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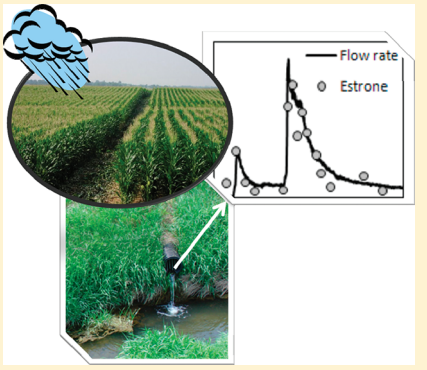
Supporting Information

ABSTRACT: Manure is increasingly being viewed as a threat to aquatic ecosystems due to the introduction of natural and synthetic hormones from land application to agricultural fields. In the Midwestern United States, where most agricultural fields are tile-drained, there is little known about hormone release from fields receiving animal wastes. To this end, seven sampling stations (four in subsurface tile drains and three in the receiving ditch network) were installed at a Midwest farm where various types of animal wastes (beef, dairy, and poultry lagoon effluent, dairy solids, and subsurface injection of swine manure) are applied to agricultural fields. Water flow was continuously monitored and samples were collected for hormone analysis during storm events and baseline flow for a 15 month study period. The compounds analyzed included the natural hormones 17α- and 17β-estradiol, estrone, estriol, testosterone, and androstenedione and the synthetic androgens 17α- and 17β-trenbolone and trendione. Hormones were detected in at least 64% of the samples collected at each station, with estrone being detected the most frequently and estriol the least. Testosterone and androstendione were detected more frequently than synthetic androgens, which were detected in fewer than 15% of samples. Hormone concentrations in subsurface tile drains increased during effluent irrigation and storm events. Hormones also appeared to persist over the winter, with increased concentrations coinciding with early thaws and snowmelt from fields amended with manure solids. The highest concentration of synthetic androgens (168 ng/L) observed coincided with a snowmelt. The highest concentrations of hormones in the ditch waters (87 ng/L for total estrogens and 52 ng/L for natural androgens) were observed in June, which coincides with the early life stage development period of many aquatic species in the Midwest.



INTRODUCTION

Estrogenic and androgenic compounds have been detected in surface waters around the world.[1−3] Humans and livestock are important sources of these compounds with major inputs to the environment including discharge from wastewater treatment plants, combined sewer overflows, and the land application of biosolids and animal wastes. The increasing size of concentrated (or confined) animal feeding operations (CAFOs) has led to manure generation at a higher mass per unit area.[4] Additionally, the amount of estrogens introduced into the environment from land application of animal wastes has been estimated to be >200 times the amount introduced from biosolids applications,[5,6] thereby increasing the potential of CAFOs to be a significant source of hormones to the environment.

Hormones associated with livestock are introduced into the environment when animal wastes are applied to agricultural fields as a nutrient source. The type and amount of hormones in these wastes vary by animal, reproductive stage, and treatment with growth-promoting compounds. Cattle excrete the majority of hormones in feces, whereas poultry and swine excrete the majority of estrogens in urine.[7] 17α-Estradiol (17α-E2) constitutes approximately 60% of estrogens in cattle feces,[1] whereas 17β-E2 and the E2 metabolite estrone (E1) comprise the majority of estrogens in poultry and swine excretions, respectively.[7] Although few studies have focused on the excretion of natural androgens by livestock, Lange et al.[5] estimated that livestock in the United States excrete 4.4 tonnes of androgens each year, with laying hens and cattle (calves and bulls) as the largest sources. Cattle receiving growth-promoting ear implants containing 17β-trenbolone acetate (TBA) excrete the synthetic androgens 17β-trenbolone (17β-TB), trendione (TND), and 17α-trenbolone (17α-TB), with the majority being excreted within the first 5 weeks after implant;[8] thus, their input into the environment is not as consistent as that of natural hormones.

Various laboratory and field studies have been conducted to assess the potential impact of CAFOs on nearby waterways. In laboratory studies, the parent hormones (e.g., E2, TB, and testosterone) have exhibited relatively short half-lives in aerobic soils and manure-amended soils on the order of a few days,[9] leading to hypotheses that hormone discharge to surface water and groundwaters should be minimal. However, Kjær et al.[10] observed hormone concentrations in tile drainage up to 11 months after subsurface injection of liquid swine manure during a 1 year

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study period. They suggested that soil temperature fluctuations and preferential flow through soil macropores may play important roles in explaining the different observations between field and laboratory studies.

Hormones are known to cause endocrine disruption in sensitive aquatic organisms at low nanograms per liter levels, although the lowest observable effect level (LOEL) varies by species and compound.[11] Definitive links between CAFO-originated hormone concentrations and altered aquatic species are complicated by the presence of other contaminants and environmental conditions; however, hormone concentrations in surface waters affected by CAFOs have been shown to be above some of the reported LOELs.[1,9] Also noteworthy is that whereas the 17β isomers of E2 and TB induce effects at much lower concentrations (<100 times lower) than the 17α isomers or other metabolites in mammalian toxicological studies, similar trends are not observed for aquatic species.[11−15] For example, 17α-E2 was found to be only 8−30 times less potent than 17β-E2 to medaka fish and fathead minnows, 17α- and 17β-TB were found to have similar reproductive effects on fathead minnow,[14,16] and E1 was found to skew sex ratios toward females and induce vitellogenin production in zebrafish at concentrations similar to or lower than those of 17β-E2.[11]

Despite the recognized negative effects of hormones on fish and other aquatic organisms, the fate and transport of hormones in agroecosystems remain poorly understood, with many studies to date being limited to experimental plots under simulated rainfall.[4,17] This study focused on hormone release from subsurface tile-drained agricultural fields at a Midwest U.S. farm where various types of animal wastes including dry manure solids, liquid manure, and animal lagoon effluent are applied to fields. Hormone concentrations were monitored in tile drains and the ditch network receiving tile drainage during storm events, base flow, effluent irrigation, and thawing/snowmelt events. The hormones monitored included 17α- and 17β-E2, E1, estriol (E3), testosterone (TST), androstenedione (AND), 17α- and 17β-TB, and TND. Hormone structures and selected chemical properties are listed in Table SI-1 of the Supporting Information (SI).

MATERIALS AND METHODS

Study Site. This study was conducted in north central Indiana at Purdue University's Animal Science Research and Education Center (ASREC), which is a working farm, an EPA-designated CAFO, and includes approximately 600 ha of tile-drained cropland. Soils at the site are predominantly silty clay loams and silt loams with loess and glacial till as the soil parent materials. Due to the presence of these poorly drained soils, perforated subsurface tile drains ranging in diameter from 10 to 61 cm were installed in the early 1990s approximately 1 m below the soil surface at 8−40 m spacings. Site maps and additional details are provided in the SI. Animal production at ASREC includes beef, dairy, poultry, sheep, swine, and Ossabaw swine units (see SI for details). Beef cattle each received a Revalor-S hormone implant containing 28 mg of 17β-E2 and 140 mg of TBA. Animal wastes are collected and stored on-site. Primary storage includes below-ground pits that are washed into above-ground lagoon systems,[18] above-ground storage units of liquid slurry from liquid/solid separators, and above-ground stacking of bedding/manure wastes. Wastes are land-applied via solids broadcasting (dewatered bedding/manure wastes), pivot irrigation (effluent from on-site lagoons), or subsurface injection (liquid slurry from above-ground storage units). Further details are provided in the SI.

Monitoring stations were installed to measure discharge and collect water samples at four tile drains (stations D1−D4) and three locations (stations S1−S3) in the ditch network at ASREC (SI, Figures SI-1 and SI-2). Each station consisted of a Campbell Scientific CR1000 datalogger, a Campbell Scientific 107 water temperature probe, a flow and/or water level sensor, a Teledyne ISCO automated sampler, and a Campbell Scientific radio and antenna enabling two-way wireless communication. Tile drains monitored by D1 and D2 are 30.5 cm in diameter, and those monitored by D3 and D4 are 61 cm in diameter. The flow rate in each tile was measured with a Marsh-McBirney Flo-Tote 3 and Flo-Station. Water levels in the ditches were monitored with a Campbell Scientific shaft encoder pulley system, and rating curves were developed to calculate flow rates.

Stations were located to capture each major animal waste application practice according to ASREC's manure management plan: (i) beef and dairy effluent (D1 and S1); (ii) beef and dairy effluent and annual applications of dairy solids (D3 and S2); and (iii) poultry and swine effluent (D4 and S3). For fields drained by D2, beef and dairy manure solids had been routinely applied up to 2007, but not during our study period. Although the major waste sources did align with our station plan, additional sources were occasionally applied to some fields. Details regarding all applications are provided in the SI (Tables SI-2−SI-8). After the start of our study, piping between lagoon systems (referred to as an interconnect system) was installed as an additional safety measure to better control lagoon heights. In addition, two valves in this interconnect system were identified to leak, thereby causing unintentional transport from north to south lagoon systems. This led to the movement of some beef wastes to the dairy, swine, and poultry lagoon systems.

Within the ditch network, S1 and S2 monitored Marshall Ditch and S3 monitored Box Ditch (SI, Figure SI-1). S1's drainage area encompassed the tile-drained area monitored by D1. S2 was located downstream of S1 and received drainage from areas monitored by stations D1, D2, D3, and S1. S3's drainage area encompassed the area monitored by station D4. Station drainage area details and total amounts of animal wastes applied are provided in Table SI-2 of the SI. Data were collected at some stations for over 2 years; however, the work presented here is focused on the data collected from January 2009 through March 2010, prior to the commencement of spring animal waste applications.

In addition to the monitoring stations at ASREC, we monitored subsurface tile drainage from a control plot at Purdue's Water Quality Field Station (WQFS), which immediately neighbors the east boundary of ASREC. This control plot (E30) has received only commercial N fertilizer for over 10 years and has never had any animal wastes intentionally applied to the field. We monitored plot E30 as a control plot from August 14, 2009, to May 16, 2010, during our ASREC study. Details are provided in the SI.

Sampling Methodology and Analysis. *Sampling Methods.* Samples were collected in 1 L polyethylene bottles using Teledyne ISCO samplers controlled by dataloggers programmed to trigger samples at time-paced intervals during base flow and at flow-paced intervals over hydrographs. Each time a sample was triggered, a 1 L sample was collected. Samples on the rising limb of hydrographs were collected at preprogrammed flow rate thresholds to appropriately capture the rise. D1 and S1 dataloggers were programmed such that real-time flow data were used to

Table 1. Data Summary Including Maximum Observed Concentrations, Percent of Total Samples Collected That Were Greater than the LOD and LOQ, and Total Number of Samples Collected (n)[a]

hormone (LOD, LOQ) (ng/L)	D1 max (ng/L) % n > LOD, % n > LOQ n	D2 max (ng/L) % n > LOD, % n > LOQ n	D3 max (ng/L) % n > LOD, % n > LOQ n	D4 max (ng/L) % n > LOD, % n > LOQ n	S1 max (ng/L) % n > LOD, % n > LOQ n	S2 max (ng/L) % n > LOD, % n > LOQ n	S3 max (ng/L) % n > LOD, % n > LOQ n
estrogens							
17β-E2	9.5	4.2	16.4	16.3	9.3	20.9	12.1
(0.06, 0.21)	33%, 19%	34%, 11%	39%, 20%	50%, 29%	39%, 23%	43%, 22%	58%, 30%
	n = 589	n = 190	n = 531	n = 552	n = 595	n = 683	n = 372
E1	18.1	1.3	25.6	33.5	23.1	40.0	9.0
(0.05, 0.16)	48%, 19%	25%, 10%	46%, 20%	78%, 41%	62%, 36%	89%, 52%	91%, 55%
	n = 589	n = 190	n = 531	n = 552	n = 595	n = 683	n = 372
17α-E2	51.8	26.7	31.1	13.3	14.1	26.9	6.1
(0.09, 0.29)	17%, 7%	13%, 8.4%	24%, 14%	18%, 11%	14%, 6.7%	29%, 11%	23%, 9.7%
	n = 589	n = 190	n = 531	n = 552	n = 595	n = 683	n = 372
E3	3.5	NA	NA	19.6	7.8	12.4	NA
(0.21, 0.64)	1.4%, 0.7%	1.1%, 0%	1.5%, 0%	2.6%, 1.1%	4.1%, 1.4%	2.5%, 1.6%	2.4%, 0%
	n = 581	n = 187	n = 522	n = 537	n = 590	n = 674	n = 368
total	68.7	27.1	53.5	42.8	23.6	87.0	12.5
	64%, 36%	27%, 23%	69%, 40%	88%, 64%	69%, 43%	91%, 60%	95%, 66%
synthetic androgens							
17β-TB	34.0	13.1	13.6	18.6	53.6	162	3.3
(0.47, 1.6)	4.6%, 1.4%	0.5%, 0.5%	4.7%, 2.0%	3.7%, 1.4%	4.2%, 2.6%	5.5%, 3.9%	2.6%, 0.6%
	n = 589	n = 183	n = 508	n = 518	n = 576	n = 506	n = 345
TND	28.5	NA	12.2	12.1	6.5	6.5	35.3
(1.9, 6.4)	3.5%, 0.7%	15%, 0%	4.0%, 0.3%	5.7%, 1.5%	3.5%, 0.2%	3.7%, 0.3%	13%, 8.5%
	n = 423	n = 142	n = 351	n = 332	n = 402	n = 377	n = 236
17α-TB	8.9	1.4	22.7	18.7	9.7	19.1	11.7
(0.22, 0.74)	6.8%, 5.6%	1.1%, 1.1%	1.4%, 1.0%	5.7%, 4.9%	3.3%, 2.6%	3.7%, 2.8%	1.4%,1.4%
	n = 589	n = 183	n = 508	n = 518	n = 576	n = 506	n = 345
total	36.8	13.4	30.1	32.7	54.3	168	35.6
	13%, 6.8%	13%, 1.6%	7.7%, 3.0%	10%, 6.7%	7.6%, 3.8%	9.0%, 4.4%	10%, 7.5%
natural androgens							
TST	12.7	2.4	3.7	16.1	50.5	15.4	11.7
(0.17, 0.58)	11%, 5.4%	31%, 10%	16%, 7%	15%, 7.9%	26%, 14%	26%, 15%	21%, 10%
	n = 589	n = 183	n = 508	n = 518	n = 576	n = 545	n = 345

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Table 1. Continued

hormone (LOD, LOQ) (ng/L)	D1 max (ng/L), % n > LOD, % n > LOQ	n	D2 max (ng/L), % n > LOD, % n > LOQ	n	D3 max (ng/L), % n > LOD, % n > LOQ	n	D4 max (ng/L), % n > LOD, % n > LOQ	n	S1 max (ng/L), % n > LOD, % n > LOQ	n	S2 max (ng/L), % n > LOD, % n > LOQ	n	S3 max (ng/L), % n > LOD, % n > LOQ	n
AND (0.12, 0.41)	1.6, 14%, 4.8%	589	2.1, 21%, 3.8%	183	8.2, 19%, 9%	508	12.5, 21%, 9.1%	518	16.6, 17%, 3.6%	576	9.4, 37%, 11%	545	5.0, 43%, 19%	345
total	14.2, 22%, 9.5%		2.4, 31%, 10% = 183		8.2, 30%, 14%		22.8, 39%, 16%		51.7, 35%, 15%		16.2, 50%, 22%		11.9, 52%, 28%	

[a] The summary is for samples collected from January 2009 through March 2010 (prior to the commencement of spring 2010 effluent irrigation). Note that due to equipment errors, data at D4 and S3 are reported only through December 2009. NA, maximum concentration not applicable; all concentrations were below the LOQ.

predict the hydrograph recession and the collection times at flow-paced intervals for the remaining samples to ensure that not all bottles were filled before the end of the recession.[19] A variation of this methodology was employed at the remaining stations with preprogrammed flow-paced intervals determined by the value of the peak flow rate such that sufficient samples would be collected during the recession for both small and large hydrographs.

Sample Preparation. Sample preparation is detailed in the SI. Briefly, water samples (1 L) were refrigerated immediately upon receipt in the laboratory for typically less than 36 h but no longer than 72 h prior to solid-phase extraction. Samples were weighed, filtered, amended with deuterated standards (6.25 ng of 17β-E2-16,16,17-d_3 and 5 ng of TST-16,16,17-d_3 dissolved in 0.5 mL of methanol), and preconcentrated by solid-phase extraction immediately or stored at 4 °C in the dark for typically less than 36 h but no longer than 72 h prior to further processing. Loaded cartridges were stored at −20 °C for up to 4 months prior to washing and eluting analytes with methanol. The eluant was evaporated to dryness, and residues were reconstituted in methanol (0.5 mL). Samples were analyzed using high-performance reverse-phase liquid chromatography tandem electrospray ionization mass spectrometry (HPLC-ESI-MS/MS).

HPLC/MS/MS Analysis. LC/MS/MS analysis was performed using a Shimadzu HPLC system coupled to a Sciex API-3000 triple-quadrupole operated in multiple reaction monitoring mode. Column and mobile phase gradient details for estrogens and androgens are summarized in Tables SI-10 and SI-11 of the SI, respectively. Retention times, precursor and product ions monitored, and the method limits of detection (LOD) and quantitation (LOQ) for aqueous samples are summarized in Table SI-12 of the SI. Method LOD and LOQ values for each hormone are also provided in Table 1 for easy reference. Other analyses performed and detailed in the SI include sample matrix effects on HPLC/ESI-MS/MS response to hormones, extraction recovery of hormones, hormone sorption to ISCO polyethylene collection bottles, and hormone stability in field samples.

RESULTS AND DISCUSSION

Hormone Recovery, Matrix Effects, and Stability. Hormone concentrations were corrected for recoveries and matrix effects using deuterated internal standards added prior to extraction and assuming similar extraction efficiencies based on similar hydrophobicities (SI, Table SI-1) and that signal suppression for estrogens and androgens could be reasonably approximated by 17β-E2-16,16,17-d_3 and TST-16,16,17-d_3, respectively. Internal standard recoveries with matrix corrections were in the range expected for large field studies with >78% of the recoveries being between 50 and 150% with an average recovery in this ranges of 91.1 ± 18.3% for 17β-E2-16,16,17-d_3 and 73 ± 19.6% for TST-16,16,17-d_3 (SI, Table SI-14). Any samples with an internal standard recovery outside the 50−150% range were included in the data analysis, but not modified by internal standard recoveries. Hormone-specific recoveries (SI, Table SI-13) and matrix effects were assessed as detailed in the SI (Figures SI-3 and SI-4). Potential errors in reported concentrations using the internal standards for recovery and matrix effects are generally within 20% (SI, Table SI-13).

Hormones are subject to sorption and microbial degradation from the time of ISCO collection to the loading of the aqueous samples onto the solid-phase cartridges. Sorption to polyethylene

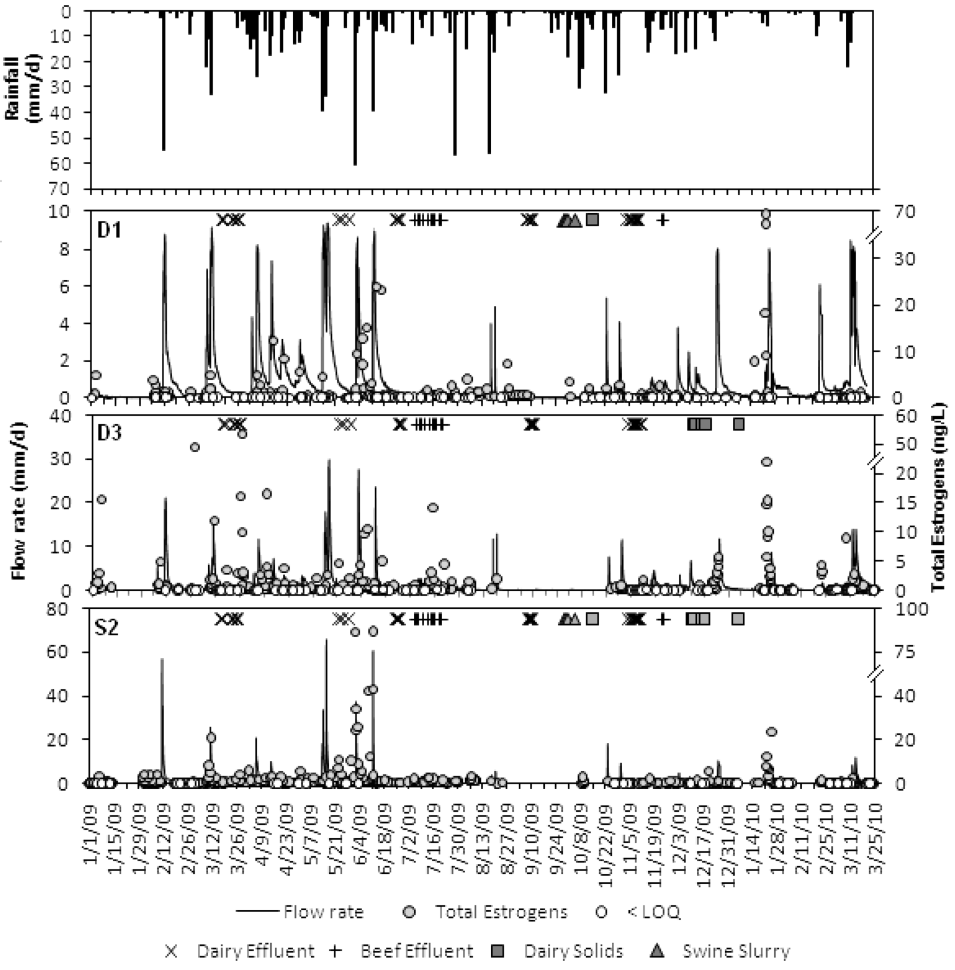

Figure 1. Hyetograph, hydrographs, and total estrogen chemographs (E1 + E2 + E3) for the study period at D1, D3, and S2. Animal waste applications are shown across the top of each panel. For graphical clarity, several high total estrogen concentrations are shown using a broken-axis notation.

bottles was assessed at 4 °C over a 72 h period (detailed in the SI). Concentrations were found to vary <5% at 0, 24, 48, and 72 h with no statistical differences at the 95% confidence level. The potential loss of hormones during sample processing was evaluated by monitoring hormone-amended S2 ditch water incubated under representative conditions, which included unfiltered and filtered stream water at 4 °C and ~23 °C. Data from stream water amended with hormones in the laboratory suggest that prior to filtering, there is a substantial degradation potential for some of the hormones within the first 24 h (SI, Figure SI-5 and SI-6), especially the natural androgens, of which up to 50% was gone within the first 24 h at 23 °C. If degradation rates estimated from the laboratory-fortified ditch water were directly applicable to field samples, then at near-steady-state conditions in the ditch network, concentrations for samples collected at later times would be expected to be higher than those from earlier collection times prior to sample pickup. However, this was not the case even for samples collected over a ~72 h period in the summer months (air temperatures of 15−30 °C). We suspect that the aerobic microbial degradation rates measured in the laboratory experiments were greatly elevated relative to the field due to aeration of the ditch water during homogenization immediately prior to hormone addition and potentially the concomitant addition of methanol (hormone carrier), which may serve as a readily available microbial food source.[20,21] Even with the expectation that degradation in our actual site samples is considerably slower than observed in well-mixed laboratory-amended ditch water, the hormone concentrations reported from subsurface tile drain and ditch network samples are likely still underestimated.

Hormone Discharge Dynamics. After land application, biogeochemical and hydrologic processes control the subsequent transport of hormones. Seasonal differences in rainfall intensity and amount, temperature, and waste management strategies confound the ability to discern between environmental and anthropogenic influences on hormone dynamics. In addition, deviations from the anticipated management plan at our study site and the intentional and unintentional routing of wastes between animal-specific lagoon systems prevent an explicit delineation of hormone release between waste types in this study. Furthermore, data interpretation of specific hormones may be biased as a result of degradation during sample collection/processing. To minimize under-representation of the hormone concentrations and associated biases, hormone levels were primarily assessed in terms of total estrogens, synthetic androgens, and natural androgens. Given the greater stability of E1 (metabolite of E2 isomers) and TND (metabolite of TB isomers), errors in total estrogens (17α-E2, 17β-E2, E1, and E3) and total synthetic androgens (17α-TB, 17β-TB, and TND), respectively, will be much smaller than the error associated with

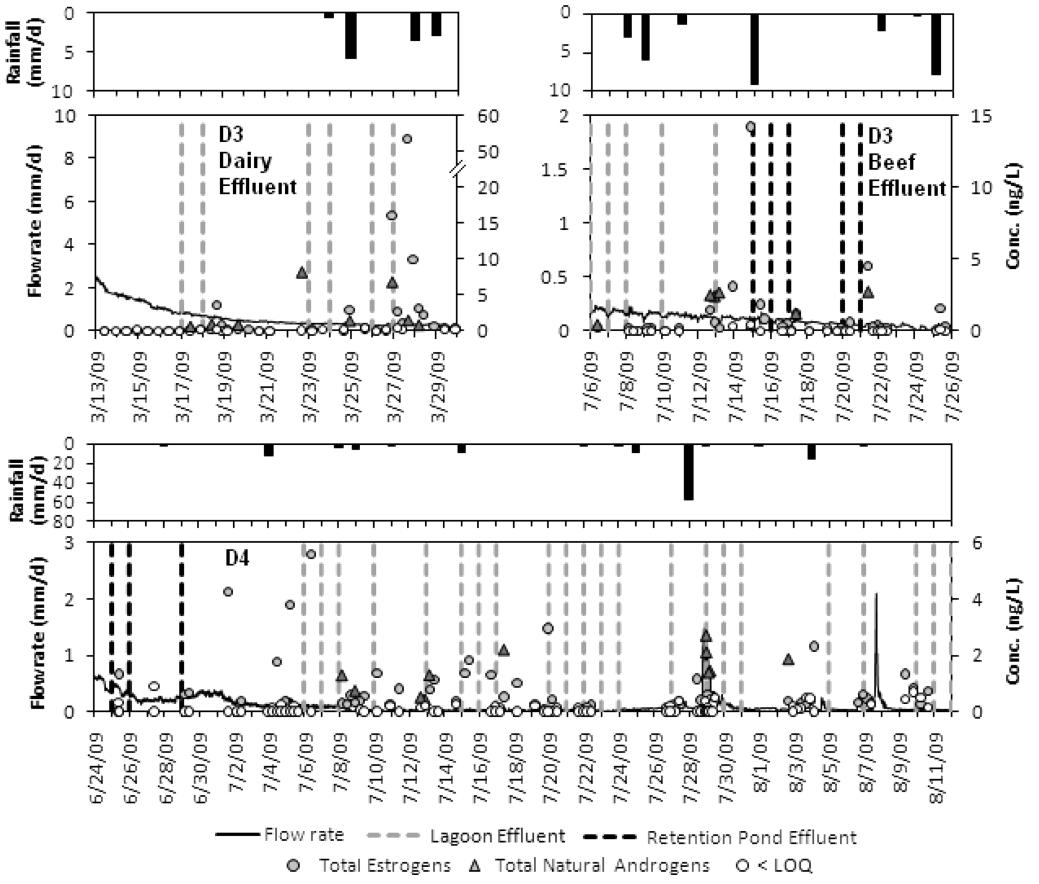

Figure 2. Hyetograph, hydrographs, and total estrogen and total natural androgen chemographs at D3 and D4. Effluent irrigation events (65.5 m^3/ha) are shown as dashed vertical lines (gray, lagoon effluent; black, retention pond effluent).

each individual hormone. Concentrations for the natural androgens (TST and AND) are likely the most underestimated because both TST and its metabolite AND degraded the most rapidly in the laboratory assessment. Using this approach, data analysis focused on trends observed during storm events, release during snowmelt events, and the influence of effluent irrigation on hormone concentrations in subsurface tile drainage. Although concentrations are likely underestimated in this study, the general trends are expected to remain the same. Given the large data set, various monitoring stations, and range of waste application types, the resulting summarization of general hormone discharge dynamics is likely to be representative of many tile-drained fields receiving animal waste applications.

Hormone Concentration Summary. The most to least frequently detected estrogens at each sampling location were E1, 17β-E2, 17α-E2, and E3, with E3 detected in <5% of samples (Table 1). Additionally, natural androgens (TST and AND) were detected more frequently than synthetic androgens (TB and TND), which were detected in <15% of the samples. Overall, hormones were detected in at least 64% of samples collected at each station that received animal waste applications during the study period. Ditch water total estrogen (E1 + E2 + E3) concentrations were highest in the spring and summer, with the maximum (87 ng/L) observed on June 1, 2009 at S2 during a 6 cm rainfall event that occurred 3 days after fields were irrigated with dairy effluent. On average, androgen concentrations were highest during the fall and winter (SI, Figures SI-7 and SI-8). The highest concentration of synthetic androgens (168 ng/L) was observed in relation to a snowmelt. However, the highest total natural androgen concentration (52 ng/L) was observed on June 25, 2009, during dairy effluent irrigation. The May−June time frame coincides with early life stage development period, a sensitive time for gonadal development and sexual differentiation, for many aquatic species.[9]

Hormone concentrations measured in the samples collected from August 14, 2009 to May 16, 2010 at the WQFS control plots are summarized in Table SI-16 of the SI. Almost all estrogen and androgen concentrations were below the LOQ with the exceptions of 17β-E2 and E1. 17β-E2 was observed in two samples with a maximum concentration of 3.13 ng/L in January 2010. E1 was observed in five samples with a maximum value of 0.38 ng/L.

Hormone Discharge during Storm Hydrographs. The influence of precipitation events on the tile drain and ditch network hydrographs is dependent on storm intensity and duration, evapotranspiration, and antecedent soil moisture conditions. During the summer months, increased evapotranspiration due to rapid crop growth and warm temperatures led to lower antecedent moisture conditions and lower tile drain and ditch flow rates relative to the winter and spring. Flow and total estrogen data collected during the study period are shown in Figure 1 for D1, D3, and S2 along with timing of animal waste applications and rainfall. Additional figures for the remaining monitoring stations and the androgens (natural and synthetic) data are provided in the SI (Figures SI-7−SI-12). Note that values in all of the hyetographs represent total daily rainfall (not intensity),

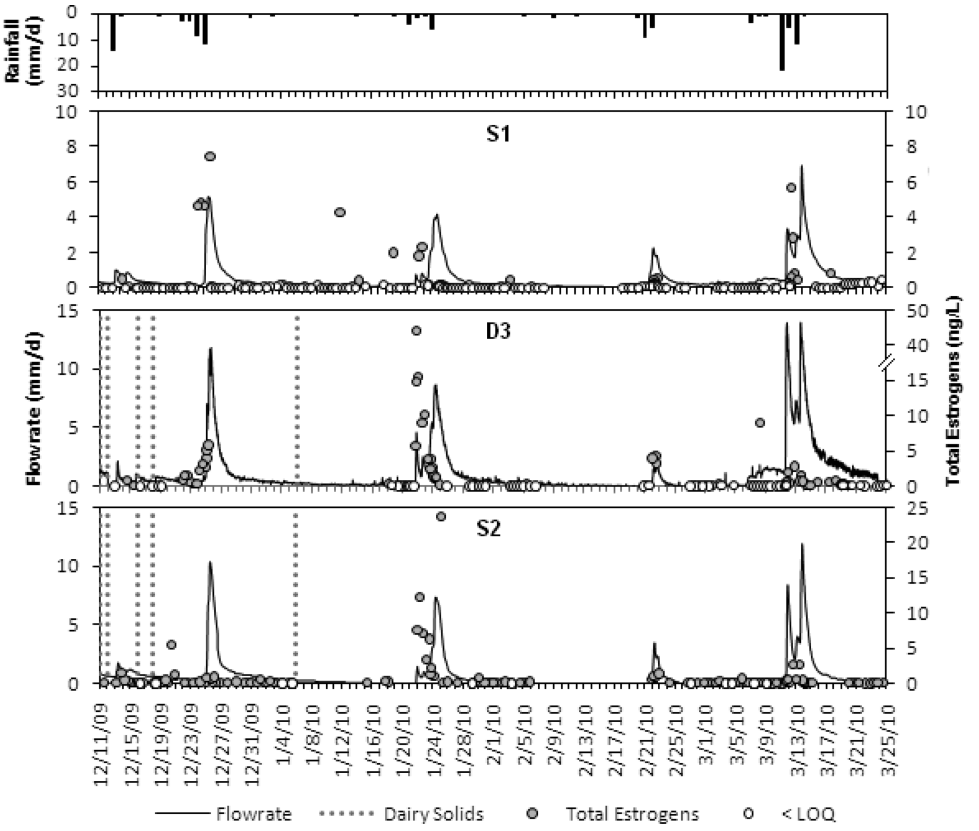

Figure 3. Hyetograph, hydrographs, and total estrogens (E1 + E2 + E3) chemographs for S1, D3, and S2 during the winter and early spring prior to the commencement of spring effluent irrigation. The timing of dairy solids applications (32.5 m^3/ha) at fields monitored by D3 and S2 are shown as dotted vertical lines. S1 received dairy and beef effluent irrigation in late November 2009 (see Table SI-6 of the Supporting Information) and is shown for comparison. Due to a sampler error, samples were missed on the recession limb of the storm hydrograph on December 23−27, 2009. Snow records indicate that snowmelt coincided with rainfall on December 22−24, 2009 (~5 cm), and February 19−20, 2010 (~8 cm). Snowmelt (~20 cm) occurred without rainfall on January 11−16, 2010.

and hormone concentrations below the LOD are plotted as 0 ng/L in all of the chemographs.

During storm hydrographs, hormone concentrations generally increased as exemplified at D1, D3, and S2 in Figure 1 for total estrogens for the study period (January 2009−March 2010). Peak concentrations were often highest during the first storm event following an animal waste application with lower concentrations observed in subsequent events before additional applications (e.g., April 2009 for D1 in Figure 1). Hormone chemographs also generally paralleled hydrographs with hormone concentrations increasing along the rising hydrograph limb, peaking near the hydrograph peak, and decreasing along the recession limb in tile drains and ditches (Figure 1 and further exemplified for D1 and D4 in Figure SI-10 of the SI). These chemograph−hydrograph similarities were the most pronounced during the first storm hydrograph following an application regardless of the magnitude of the peak storm hydrograph flow rate. The steep rising limb of tile drain hydrographs is due primarily to macropore flow, which is known to transport land-applied chemicals to receiving ditches, especially in the first two rain events after application.[22]

Transport of hormones sorbed to soils or associated with manure solids (see Table SI-1 of the SI) are also highly subject to surface runoff during high-intensity rain events. Although surface runoff was not measured directly, it was inferred to occur when flow in the smaller tile drains (e.g., D1) reached full capacity and the area-normalized flow rates were substantially higher in the ditches than in the larger tile drains (e.g., D3). Intense rains occurred several times in May and June 2009, leading to full-capacity tile flow in D1 and surface runoff to the ditch network (e.g., D1 and S2 in Figure 1 and detailed in Figure SI-11 of the SI). During the first two storm events in June after dairy effluent irrigations, total estrogens (Figure 1 and Figure SI-11 of the SI), natural androgens (SI, Figure SI-7), and synthetic androgens (SI, Figure SI-8) in ditch water increased to levels above those observed in the tile drains with concentrations highest at S2 (downstream of S1). Surface runoff is typically high in suspended solids, to which hormones may be sorbed. Hormone concentrations at D2, which drains an area that had not received manure applications since 2007, also increased during these events (SI, Figure SI-12).

Rapid Transport following Effluent Irrigation. Effluent irrigation is typically used during late spring and summer while crops are growing and evapotranspiration rates are high; however, to minimize the potential of lagoon overflow during periods of snowmelt and heavy spring rainfall, effluent was also frequently applied in March and November (see Tables SI-3−SI-8 of the SI). In general, hormone concentrations following effluent irrigation increased during rainfall events as exemplified in Figures 1 and 2. Additionally, hormone concentrations occasionally were observed to increase during and shortly after effluent irrigation events that were not associated with rainfall. For example, hormone concentrations

increased on March 18 and 27−28, 2009 at D3 following dairy effluent irrigation, July 12−13, 2009 at D3 following beef effluent irrigation, and during several poultry effluent irrigation events during July and August 2009 at D4 (July 12−13, 17, and 27; August 6 and 10) (Figure 2). Elevated concentrations, albeit low nanograms per liter, were also observed at D1 during July 2009 irrigations (Figure 1). These trends were more pronounced when effluent irrigations occurred shortly after rainfall, which increased antecedent soil moisture. Higher antecedent soil moisture conditions have been correlated to enhanced macropore flow of chemicals,[22,23] although not consistently.[24] Effluent irrigation also influences soil moisture conditions, as each irrigation (65.5 m^3/ha) was the equivalent (in terms of moisture) to ∼6.6 mm of rainfall. In some cases, hormone concentrations in tile drainage were higher during effluent irrigation than during rainfall events (e.g., March 26−28 at D3; July 12−13 at D3; July 12−13 and 17 at D4). Hormone concentrations in tile drains immediately following effluent irrigation appear to be indicative of preferential flow through an established macropore network. The latter is consistent with subsurface tile drainage studies in which tracers in irrigation water reached the tile drain within 1 h after irrigation regardless of their sorption characteristics.[22]

Although no direct measurements of preferential flow were made at the study site (e.g., tracer studies), preferential flow is known to occur at similar subsurface tile-drained fields and has been observed within tracer studies at experimental tile-drained plots at the Purdue WQFS immediately adjacent to ASREC.[25] These observations of rapid solute transport to subsurface tile drains are consistent with observations at several other field studies.[26−29] Notably, Lapen et al.[27] observed "application-induced discharge" (as opposed to "precipitation-induced discharge") of pharmaceuticals and personal care products to tile drains following land application of biosolids with concentrations increasing within minutes after application. They attributed this rapid transport to flow through networks in the soil that directly connect to the tile drains (i.e., preferential flow pathways). Transport through such networks reduces the reactive time with soil particles, potentially reducing sorption and degradation[30] and increasing the potential importance of preferential flow to water quality implications with regard to hormones.

Hormone Preservation and Discharge during Cold Months. According to best management practices, solid manure applications should occur when soil temperatures drop below 10 °C to minimize the potential for nutrient loss;[31] however, colder temperatures also can preserve manure-borne hormones. When temperatures rose in early February 2009 (>13 °C) and caused a snowmelt (∼5 cm of snow was on the ground at this time), total estrogen concentrations increased to 12 ng/L at D3 (Figure 1), for which the last waste application had been dairy solids in September 2008. Estrogen concentrations also increased during a large rain event (total rainfall of 6.5 cm over a period of 4 days) in early March 2009 at both D3 and S2 prior to the commencement of spring effluent irrigation (Figure 1). Total synthetic hormones also increased at D3 and S2 during this event, with S2 reaching a maximum value of ∼170 ng/L (SI, Figure SI-8). Fields drained by D1 and S1 did not receive solid applications but were irrigated multiple times with dairy and beef effluent in fall 2008 (SI, Tables SI-3 and SI-6). Additionally, estrogen concentrations increased at D1 during the February snowmelt and early March rain event, although concentrations were higher at D3 and S2 (Figure 1). Total synthetic hormone concentrations also increased at D1, D3, and S1 during the February event (SI, Figure SI-8). These observations suggest that hormones are preserved in the field during the winter months and that fall applications of solids lead to greater winter and early spring export of hormones than fall effluent irrigation.

Similar trends were observed at D3 and S2 in winter 2009 and early spring 2010 (Figure 3). Fields monitored by D3 and S2 received several applications of dairy solids later in the year in 2009 than in 2008, with one application occurring in early January 2010 when ∼5 cm of snow was on the ground (SI, Tables SI-4 and SI-7). Fields monitored by D1 and S1 received one application of dairy solids in early October 2009 and multiple applications of dairy and beef effluent (SI, Tables SI-3 and SI-6). Total estrogen concentrations increased during each storm event following these applications (Figure 3) through March prior to the commencement of spring effluent irrigation. Hormone concentrations increased to higher values at D3 and S2 than at S1 during the rain event on January 22−28, 2010, likely due to the recent dairy solids applications. The apparent preservation of hormones exemplified three times at the site (early and late 2009 and early 2010) suggests that such winter and early spring dynamics play a significant role in hormone export and can be expected at other subsurface tile-drained sites.

Implications and Study Limitations. Subsurface tile drains are well-known to change the pore structure within soil profiles, dramatically altering the natural hydrology and expediting the transport of water and solutes through the soil profile, into the tile drains, and ultimately into nearby surface water bodies.[22] However, the role these systems play in hormone discharge following land application of animal wastes is not well-known. Rapid increases in hormone concentrations observed in tile drains following effluent irrigation suggest aqueous and particulate-borne hormones are rapidly transported to subsurface tile drains through an established macropore network consistent with tracer irrigation studies.[22] During storm events, the rise and fall of hormone concentrations in tile drainage generally followed hydrograph trends. When smaller tile drains flowed full and area-normalized ditch flow rates increased significantly compared to flow in the larger tile drains, elevated hormone concentrations in the ditch network relative to tile drainage suggested hormone transport via surface runoff. Peak hormone concentrations in the ditches occurred in June shortly after effluent irrigation, coinciding with a sensitive early life stage development period for many aquatic species. Cold temperatures during the winter months appeared to preserve hormones from late fall animal waste applications and resulted in increased hormone export during storm events via tile drainage to the ditch network in the early spring. This increase in hormone concentrations during storm events continued for as long as 4 months after fall animal waste applications. Winter rain events and snowmelt increased exported hormone concentrations three times during the 15 month monitoring period (early and late 2009 and early 2010), suggesting that hormone export through such winter and early spring dynamics may be expected at similarly managed subsurface tile-drained sites.

The ASREC site presented a unique opportunity to evaluate the discharge of hormones following various animal waste applications occurring at similar field study sites, with each animal waste type applied multiple times during the study period. Although the study did add to our understanding of the potential contribution of hormones from animal-derived effluent and solid

wastes applied to subsurface tile-drained fields, the management complexity of the site, including multiple types of wastes applied to a single drainage area, limited explicit interpretation of the data collected. Sufficient characterization of the waste being applied was also limited due to the challenge of obtaining waste samples in a regular and timely manner given the application frequency and more pressing responsibilities of farm personnel. In addition, identifying some level of sample preservation that did not interfere with hormone analysis would have helped to minimize underestimation of the discharged hormone levels. Finally, real-time monitoring of soil moisture and several key surface runoff collection points would have improved the utilization of the data set toward recommending improved animal waste management strategies.

■ ASSOCIATED CONTENT

Supporting Information. Study site details and figures, additional information regarding sample analysis, and additional figures depicting hormone chemograph behavior. This material is available free of charge via the Internet at http://pubs.acs.org.

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